DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

FOREST PRODUCTS RESEARCH

BULLETIN NO. 4

A DECAY OF SITKA SPRUCE TIMBER, CAUSED BY *TRAMETES SERIALIS*, FR.

A CULTURAL STUDY OF THE FUNGUS

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PREFATORY NOTE

A COMPLETE knowledge of the fundamental causes of decay in timber is of primary importance, and extensive investigations are now being undertaken at the Forest Products Research Laboratory at Princes Risborough. They may be grouped under four heads, namely, the mycological aspect, to ascertain not only the fungi which are the cause of decay, but particularly the way in which they penetrate and grow in wood tissues and the surroundings necessary for their activities; the chemical aspect, to investigate the chemical changes which may take place as growth proceeds; the mechanical aspect, to ascertain the general effect on the strength of the timber as the fungus develops; and, finally, a critical examination on the anatomical structure of the timber at the beginning of and during the process of decay.

A peculiar defect in timber, resulting in abnormal fracture taking place when it is subjected to load, is thought to be due to a combination of causes primarily set up by fungus attack. It is found to be present in certain species more than others, such as in ash, Sitka spruce and many tropical woods, and it is a characteristic most difficult and necessary to detect, particularly in timbers required for use in shafts, tool handles, beams and aircraft construction.

The bulletin now submitted is the record dealing with one aspect of the mycological side of the question, and is a step forward in the general advance of research into a difficult and complicated problem.

Grateful acknowledgments are due to Professor Percy Groom, F.R.S., for his help and advice during the progress of the work; to Miss Irene Mounce and to Dr. Audrey Richards, who so kindly sent cultures, and to Mr. Tooley for the time he gave to the photographic illustrations. Finally, thanks are due to Mrs. Cartwright for her able execution of many of the drawings.

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A DECAY OF SITKA SPRUCE TIMBER, CAUSED BY *TRAMETES SERIALIS*, FR.

A CULTURAL STUDY OF THE FUNGUS

INTRODUCTION

THE investigation to be described was inaugurated at the request of the Air Ministry, owing to the large percentage of Sitka spruce timber which was found on arrival from Canada to be unfit for use for aircraft construction, due to various defects, some of which were suspected to have been caused by fungal agency.

In spite of stringent inspection on arrival it was found impossible to detect all faulty material and thus prevent it passing into store, and in 1925 it was noticed that, at one of the storage depôts, a large percentage of the timber showed signs of deterioration and was unfit for use.

Professor Percy Groom, F.R.S., was thereupon consulted and was invited to inspect a batch of timber at this depôt. He reported that, in addition to sapwood stain, which was very prevalent, there were symptoms, in some of the samples examined, pointing to more serious decay of an internal nature, and advised that an investigation should be undertaken. This was agreed to by the Department of Scientific and Industrial Research, and the inquiry was entrusted to the writer.

A preliminary survey was accordingly carried out in December, 1925, at the depôt from which infection was reported, and sufficient material collected for an intensive study. All the material was obtained from one locality.

The inspection at the depôt showed evidence of a good deal of sapwood discolouration, mostly the characteristic " Blueing " caused by *Ceratostomella piceæ*. In addition, a number of moulds were present, the one of most frequent occurrence being the fungus *Trichoderma lignorum*, causing a yellow to pinkish-orange discolouration, not confined entirely to the sapwood. Apart from the above, there were only a few samples showing obvious signs of advanced decay, and no external growths of wood-destroying fungi were observed. One sample showed black zone lines at the junction of attack between two fungi ; these areas, delimited by the zone lines, were spherical in outline on the transverse face of the block.

In by far the majority of cases the wood seemed, at first sight, to be sound, and the moisture content did not appear to be unduly high.

On testing with a knife, however, much of the wood proved to be brittle, the fibres breaking off short, and in some cases a slight yellowish to pinkish-purple discolouration was observed, as a rule in pockets, running longitudinally.

On hand sectioning, in certain cases, a few fine hyphæ could be detected. In many cases the wood appeared to show no signs of the presence of any fungal elements, though on closer microscopic examination their presence could be detected, often in samples where in the preliminary examination no discolouration had been observed and no hyphæ had been located.

METHODS USED FOR ISOLATING WOOD-DESTROYING FUNGI

Various methods of isolating lignicolous fungi and getting them into pure culture have been carried out, such as :—

(*a*) A portion of a block was split and a section taken with a sterilized razor, this section being transferred to a slant of suitable medium.

(*b*) Thicker portions were taken and surface sterilized, either by charring or flaming the exposed surfaces after treating with absolute alcohol, these being then half buried in the medium.

Neither of these methods proved entirely satisfactory for the isolation of wood fungi, whether from the living tree or from converted timber, and finally the following method was employed :—

(*c*) A Pressler borer (an instrument used by foresters to extract plugs of wood for the measurement of annual growth) was used to obtain a plug of wood from the interior of the block. The depth from which the plug was taken depended upon the state of decay of the specimen from which the culture was required. The greatest percentage of success was obtained when the plug was taken from the margin of attack, or at any rate from wood that did not show any appreciable sign of disintegration. In the case of cultures taken from logs showing fruit bodies, the most satisfactory method was to take the boring, where practical, through the tissue of the sporophore into the wood just behind this layer and at the back of it. The outer portion of the plug was then removed and the inner portion alone utilized, which consisted of wood with no portion of the fruit body. In many cases the sporophore tissue can be successfully used, but it has been found occasionally, in woody species, that the tissue either refused to grow unless the sporophore was in an early stage of development, or contaminants were present, which, being the more rapid growers, prevented the growth of the sporophore tissue. In the case of more fleshy species, such as many of the annual *Polyporaceæ* and *Agaricineæ*, bacterial contaminants often caused trouble.

Using the above method, it has been found possible to obtain pure cultures in about 95 per cent. of cases, even where plugs from other places in the same sample may have given very mixed cultures. Where possible, check cultures have been made, using portions of sporophore tissue and also cultures made from spores ; but in many cases, especially in some *Fomes* spp., the tissue failed to grow and the spores in many of the species are few and often difficult of germination. In all cases the outer portion of the plug was removed with a sterile knife, the piece remaining being partly buried in the medium.

SPECIES OF FUNGI ISOLATED

From the material collected at the depôt the following four different fungi belonging to the *Basidiomycetes* were isolated, the one (i.e. *Trametes serialis*) dealt with in this paper occurring on about 90 per cent. of the samples collected.

i) A fungus which in culture resembled closely *P. Schweinitzii*, and is thought in all probability to be a form of that fungus, differing slightly in colour range and in the pronounced odour of the young culture. (This fungus has also been isolated by Miss Irene Mounce, working independently on this problem in Canada.)

(ii) A fungus which forms numerous bulbils and whose mycelium showed numerous clamp connexions, was obtained from one block which appeared to have a larger proportion of sapwood than the majority of the samples. This was identified as *Corticium alutaceum* [1, 2]*, now transferred to *C. radiosum*.

(iii) A fungus which formed sclerotium-like bodies, probably of the nature of abortive sporophores, was also isolated, and is thought to be an *Exidia*, but the identification is not sure.

None of the above fungi, with the possible exception of *P. Schweinitzii*, was considered to be the chief cause of the decay.

(iv) The fungus *T. serialis*, with which this paper deals, was obtained from many separate isolations, attacking both sapwood and heartwood. Later, after the samples had been kept under favourable conditions of moisture, external evidence of its prevalence was obtained by the appearance of mycelial strands and fruit bodies.

Microscopic examination of the wood had shown fine hyphæ bearing both simple clamp connexions and the medallion type of clamps, the latter much more sparingly. Also, in places, round swellings were observed on some of the larger hyphæ. These hyphæ were, when older, yellow in colour and possessed thick walls. From the above characteristics the fungus was in the first instance thought to be a *Lenzites* species, and further evidence apparently in favour of this was found both in its temperature relations and also, later, in the formation in culture of a thelephoroid type of fruit body [3]. This identification, on further cultural study, proved to be erroneous.

IDENTIFICATION OF *T. SERIALIS* AS A CAUSE OF SITKA SPRUCE DECAY

The identification of the fungus proved difficult and was complicated by the fact that the material came from Canada, making it probable that infection took place in that country, and thus widening the possible number of fungus species responsible for the decay. Little work had been done on the identification of these wood-destroying fungi in this country, and though useful work is being carried out in Canada at Ottawa [8] and in the United States at Madison, which has proved of great value to the writer, no description which agreed with cultures of this fungus was found.

When the investigation was well advanced, through the good offices of Dr. Butler, of the Imperial Mycological Bureau, Kew, the writer was put in touch with the Dominion Botanist, Dr. Güssow, of the Central Experimental Farm, Ottawa, under whose direction work on the same problem of infected Sitka spruce was being undertaken by Miss Irene Mounce. Cultures were exchanged, and it was found that several of the fungi isolated by the writer had also been obtained from the spruce on the Canadian side. Amongst these was the fungus considered to be a form of *P. Schweinitzii* and the fungus dealt with in this paper, the latter, however, not having been identified. Reference was made to many papers, in which were descriptions of abnormal fruit bodies, and in a paper by W. H. Snell [9] a brief description of *T. serialis* in culture was given. This paper referred to the formation of foliose fruit bodies, and it appeared probable from this and from the fact that the rot agreed with that described by Hubert [10] for this species (i.e., *T. serialis*), that this was the fungus isolated by Miss Mounce in Canada and, independently, by the writer in this country.

Type cultures were kindly provided by Dr. Audrey Richards, of Madison, and these proved beyond doubt that the fungus isolated by the writer was identical with *T. serialis*.

* These numbers refer to the Bibliography on p. 20.

EXTERNAL APPEARANCE OF *T. SERIALIS* ON ORIGINAL WOOD SAMPLES

The blocks were soaked in water overnight and put into potato dishes, with a small container of water in each, in order to retain a moist atmosphere. No external evidence of the fungus appeared on any of the blocks until many months had passed, and in several cases it was not until after fully eighteen months that there was any appearance of external mycelium.

Strand formation.—The first external appearance of the fungus was in the form of white strands, somewhat resembling those seen in wood cultures of *Poria vaporaria* obtained from the Centraal Bureau voor Schimmelcultures, Baarn (Plate I, fig. 1). These strands remain white or may turn a yellow to ochre colour. The strands varied in thickness, but in most cases were of a considerable diameter (500 μ) and branched out into white tufts of mycelium, which grew downwards towards the moisture supply. The appearance of this external mycelium depended entirely upon an abundance of moisture, none appearing on blocks kept under dry conditions.

Structure of external strands.—The strands were composed of a central core of coloured, thick-walled hyphæ surrounded by an outer thin layer of fine white hyphæ. The thick-walled hyphæ were irregular in outline, many swellings occurring, which varied greatly in size, forming chlamydosporic structures. The hyphæ showed frequent fusions, strands formed of many hyphæ appearing as if enclosed in a common wall. The clamps in these coloured hyphæ were, in the case of those most deeply coloured, difficult to observe, as they became occluded by the thick coloured wall; but all stages of inclusion of clamps were found, though in certain cases all that could be seen was a refractive spot. It was noticed that the clamps often appeared to occur in conjunction with a swelling, the swelling including a clamp. (Plate VI, figs. 1–5.)

Fructifications.—In some of the drier blocks, white cushions of about ½ in. in diameter appeared. These turned cartridge buff, pale ochraceous salmon, cinnamon brown to buckthorn brown* on ageing, and also became discoloured on bruising, thus showing the same colour range as seen in the mycelium of old cultures. The cushions were usually elongated in a longitudinal direction, developing pores which later were torn into lamellæ or teeth, approaching the Irpex form of hymenial surface. A normal hymenial surface was formed with basidia bearing four spores on sterigmata; these spores germinated readily in drop cultures. Similar fructifications appeared later on the moister blocks as the moisture decreased. Fructifications of varying types are shown on Plate I, figs. 3 and 4, and Plate II, figs. 1 and 2.

In other cases, white to yellowish thelephoroid-like fructifications were formed, all stages from the thelephoroid, hydnoid, poroid, irpiciform and, in rarer cases, dædaleoid types being found.

Mycelium.—Examination of the mycelium from sections taken from the interior of the blocks showed extremely fine colourless hyphæ often invisible until stained. These hyphæ ran for the most part longitudinally and were often closely adpressed to the cell wall. Branching occurred at intervals and a similar mycelium was found sparingly running through the cells of the medullary rays. The mycelium appeared to occur in isolated pockets running longitudinally, these pockets being connected by a sparse growth of fine hyphæ which ran both in a transverse and longitudinal direction.

* All colour nomenclature employed in this paper has been taken from Ridgway's colour standards.

In places where the fungus hyphæ were plentiful there was much erosion of the bordered pits, and bore-holes were present both in the pit-walls and in the walls of the tracheids ; these bore-holes were large and in some cases, though not invariably, tended to be hour-glass shaped, the middle lamella appearing to be slightly more resistant to fungus action. (Plate III, fig. 10.)

Clamp-connexions were found sparingly on the hyphæ in the wood, these being of the simple unbranched type. On certain of the hyphæ the medallion type of clamp occurred, which appears to be really in the nature of a double clamp. (Plate III, fig. 5 ; Plate IV, figs. 1 and 2.)

It was found that the usual stains such as cotton blue or methyl violet and bismark brown were only successful in cases where there was an abundance of hyphæ of large diameter present and the details of attack could not be easily observed by their use ; the staining was often very uneven. Good differentiation was often obtained by the use of Pianeze 111B, the mycelium staining pink against a bluish-green background, but even this stain appeared to give somewhat uncertain results and was not permanent. Eventually a combination stain was evolved which showed up even the finest hyphæ and minute details of their penetration through the cell walls, namely, aqueous safranin and picro-anilin blue. This same stain has been tested on many other wood-inhabiting fungi, including the difficult *Stereum purpureum*, and has been found satisfactory in every case. A note on this method of staining has been published.[4] (See also Appendix, p. 21.)

CULTURES ON WOOD

Pure cultures were obtained by the use of Pressler's increment borer as described on p. 2 (*c*), the plug being partially buried in a slant of 2 per cent. Kepler's malt agar-agar. Cultures were also obtained, later, on the formation of sporophores on the original wood blocks, from pieces of the fruit body tissue. Single-spore cultures were also made. These monosporous cultures developed no clamps for a week or so. After this initial period, in certain of these cultures clamps were formed, whilst others remained in the primary condition. It appears, therefore, that this fungus affords yet another instance of a basidiomycete with " sex " complications similar to that worked out for *Coprinus* by Hanna[5] and for *Schizophyllum* by Kniep[6].

Sterilisation.—Some blocks of Sitka spruce, 1 in. by 1 in. by 5 in., were placed in boiling tubes, a wad of absorbent cotton wool being placed at the bottom. To these, varying amounts of water were added and the tubes autoclaved at a pressure of 15 lb. for 20 minutes. Others were intermittently sterilized in a steamer for one hour on three successive days and other blocks were charred on the surface or flamed with alcohol, but it was found that the last two methods were not entirely satisfactory.

An examination of the blocks decayed by the fungus showed that the rate of attack was not materially altered whether the blocks were autoclaved or steamed, though this has not been found to be invariably the case when dealing with wood-attacking fungi.

In consequence of the above preliminary trials, all material in the present instance was autoclaved, this method saving much time and ensuring sterile conditions.

Inoculation, and notes on subsequent growth.—The blocks were inoculated with small portions taken from the malt agar cultures, the portion removed being placed on the side of the wood block at a distance of about $\frac{1}{2}$ in. from the base of the tube. In some cases the cotton wool plug was covered with cellophane to prevent evaporation,

and this covering was occasionally removed to allow for aeration. In others no covering was used. It was found that the most rapid growth was obtained with a moisture content between 45 per cent. and 65 per cent., this being well above the fibre saturation point of the wood. At higher moisture contents, growth tended to be confined to the surface. A temperature of about 25° C. caused a marked increase of growth, but as it was desired to study the action of the fungus under normal conditions, the tubes were for the most part kept at laboratory temperature.

In the case of the tubes the plugs of which were covered, after some months the visible growth was almost entirely confined to the top end of the tube. Generally, growth started fairly rapidly and a sparse network was formed over the surface of the block, being more profuse at the top than at the base. As the surface of the block became drier, external growth diminished, and this was the stage at which the wood began to show serious signs of attack. Later, the wood became darker in colour, the discolouration tending to occur in longitudinal streaks, and soon afterwards strand formation was observed as in the original blocks. At this stage the thelephoroid or irpiciform fructifications often appeared on the sides of the block (Plate I, figs. 3 and 4), and in one case in which the plug had been covered over, these fructifications were formed at the top end and were slightly stipitate in form. (Plate II, fig. 1.)

Cultures also were made on Sitka spruce sawdust, and these fruited readily, the form being usually of the irpiciform type. (Plate II, figs. 3 and 4.)

The rate of decay varied considerably according to the conditions, but, when optimum for temperature, aeration and moisture, wood destruction proceeded rapidly. Although its growth appears to be susceptible to variations of moisture content, the fungus was found to be resistant to desiccation, as, after having remained dormant for some time, it was possible to bring it into active growth on favourable conditions being restored. How long the fungus may remain alive in air-dried timber with a moisture content of between 10 per cent. and 12 per cent. is uncertain, but undoubtedly for a considerable period. It is highly probable that this power is possessed by all those fungi forming secondary spores of the chlamydospore type, and consequently these species should be regarded as amongst the most dangerous of the wood-destroyers.

Sections were prepared from the blocks after varying periods from the time of inoculation. For the preparation of longitudinal sections it was found unnecessary to employ a softener, as they could be easily cut with the aid of a Jung microtome without further preparation.

The first visible sign of decay was the appearance of streaks of discolouration, usually seen on splitting open the sample. In some cases the wood appeared to be water-logged in patches; at a later stage it tended to crack in longitudinal lines, and in the final stages, on the drying up of the wood, it became cracked at the surface into minute cubes, the smaller cracks occurring at right angles to the original and more pronounced longitudinal fissures. (Plate I, fig. 2, No. 4.) At this stage the wood becomes very friable, although there may be still an appreciable amount of strength left in the summer wood.

MICROSCOPIC STRUCTURE OF THE FUNGUS IN ARTIFICIALLY-INFECTED BLOCKS

The hyphæ, which are for the most part of small diameter, run longitudinally through the tracheids, branching at rare intervals and sparsely septate. They adhere closely to the walls of the tracheids, and in early stages there is no sign of any attempt at penetration into the cell walls, neither do they pass through any pits for a considerable distance.

Clamp connexions, usually of the simple type, occur here and there, but in this early stage no medallion type of clamps have been observed.

The first internal sign of attack was a slight erosion of the walls of the bordered pits and the attack on the tracheids appeared to start from the lumen. At a later stage, the fine hyphæ penetrated the walls of the tracheids and also those of the bordered pits indiscriminately, but rarely were the hyphæ observed to pass through the pit lumen. The bore-holes were large, often appearing two to three times as large as the hyphæ passing through them. Only in a few instances were hyphæ of large diameter seen to penetrate the walls, and then the holes were slightly of the hour-glass shape, though this feature was not so well marked as in many other wood-destroying fungi. The hyphæ might or might not show constriction when passing through a cell wall; in certain instances they were seen to taper somewhat abruptly when still at a considerable distance from the tracheid wall. A fuller description of the bore-holes will be found on page 8.

The hyphæ of large diameter were for the most part situated in the summer wood, and were also observed in later stages to be connected with chlamydospore formation.

In advanced stages, thick-walled hyphæ, coloured orange-brown, were found in tracheids of the summer-wood. These coloured hyphæ possessed frequent septa and were much branched. They reacted to the safranin and picro-anilin blue stain in a different manner, often not staining at all, but more frequently taking up the safranin to a certain extent, thus showing that either they had lost their living contents or that, at any rate, the contents had become chemically altered.

The tracheids were often entirely filled with these large type of hyphæ, which in young stages, before they became coloured, showed up as twisted masses of blue with picro-anilin blue dye. These masses ran in longitudinal pockets, thus giving the external signs of streaks described earlier. Chlamydospore formation was profuse in these pockets, and eventually the hyphæ, from which they had been cut off, disappeared, thus leaving isolated chlamydospores. (Plate V, figs. 1, 2 and 3.) Further, the mycelium in these pockets bore clamp connexions in abundance, and the medallion type, though less abundant, also occurred.

In badly decayed wood, diagonal cracks were numerous. These appeared to be rather irregular, and cross cracking was never seen as in tracheids attacked by *P. Schweinitzii*. The pits also showed cracks radiating out from the centre, and, in the most advanced cases of attack, the whole pit had become entirely eroded away.

When wood attacked by the fungus was tested with phloroglucin and HCl, there was little, if any, diminution in the intensity of the resulting colouration as compared with that in sound wood. Cellulose tests, such as Schultze's, are masked by the lignin present, so that it is difficult to demonstrate the reaction.

However, in testing for enzyme action, wood sections left in fungus extract and incubated were compared with similar sections left in water and in autoclaved extract. Tests showed that the fungus was capable of utilizing cellulose or portions of the cellulose complex. As the rot is of the so-called brown type, this is what was to be expected.

It has been shown by chemical analysis at the Forest Products Research Laboratory that *T. serialis* is capable of destroying the cellulose in Sitka spruce sawdust[11].

Measurements and Microscopic Details

Hyphæ.—The fine colourless hyphæ measured from 2 to 4μ. Septa occurred at rare intervals and clamps of the simple type were frequent; the medallion type was also observed. The coloured, thicker-walled hyphæ (present mostly in the summer

wood) measured from 3 to 9μ in diameter, and in these the septation was more frequent. Their frequency, however, varied too much for measurements of the individual units to be of any diagnostic value. On these coloured hyphæ clamp connexions were present, though often difficult to observe, owing to the fact that they were generally found occluded in the coloured thick wall as described earlier.

Chlamydospores.—Irregular swellings were numerous on the coloured hyphæ, and these varied considerably in size and shape. They might be spherical, ovate or more often spindle-shaped, and in some cases were by no means symmetrical. They possessed thick walls, and their contents stained deeply with the picro-anilin blue; the wall either remained colourless or at times took up the safranin stain to a certain extent.

Their average size varied from 10 to 20μ when spherical, the length being more often slightly greater than this in those which were spindle or ovate in shape, in which case their transverse diameter was proportionately less, i.e. (15 × 12μ, 10 × 6μ, 8 × 7μ, etc.). (Plate V, figs. 1, 2 and 3.)

These spores eventually became isolated from the mycelium through the shrinkage, and finally the total collapse of the hyphæ on which they had been borne.

Their origin was both intercalary and terminal, and often they were formed in long chains so that, on the death of the intermediate portions of the hyphæ, the tracheids were filled with these thick-walled spores, the contents of the hyphæ from which they had been formed having apparently passed into them.

Fruit bodies.—The most usual form observed has been the thelephoroid type. (Plate I, fig. 3, etc.) These consisted of loose plates ranging through the following colours: Cartridge buff, pale ochraceous salmon, cinnamon brown. They darkened in colour on ageing or bruising. Irpex, hydnum, dædalea and poria types also occurred. All fruit bodies examined have been found to bear a normal hymenial surface with basidia, measuring on an average 12 to 15μ × 3·5 to 5μ (occasionally 18μ × 6μ). Each basidium bears two to four spores, measuring 5 to 6μ × 2·5 to 3μ, borne on sterigmata which are 2μ to 5μ in length. (Plate V, fig. 5.)

Bore-holes.—The bore-holes were large and fairly uniform in size, being 4μ to 5·5μ in diameter. They occurred in the walls both of the pits and tracheids.

Penetration.—It was notable that, although a diligent search was made for early stages of incomplete penetration, no definite case of this was seen, though three probable cases are figures in Plate III, figs. 1, 2 and 3.

In a few cases it appeared that the middle lamella was still intact, and in others that the hole only penetrated partially through the wall. On closer observation, however, these latter cases seemed to be more satisfactorily accounted for by the fact that the section had not passed directly through the median plane of the hole. The sides of the bore-holes were in all cases smooth and clean-cut. These facts could only be explained by the hypothesis that either actual penetration took place with extreme rapidity, or, alternatively, that the hole had been made with the aid of chemical action in advance of the hyphæ. It is, at any rate, likely that the resistance is broken down in advance to such an extent that the final penetration can be speedily accomplished.

The latter suggestion appears in the opinion of the writer to conform to the greatest extent with the observed facts. Further evidence for this view will be discussed hereafter, and it more easily explains the regular outline of the holes, which would hardly be expected if the action were entirely of a chemical nature in advance of the hyphal

tip. The process of penetration, as far as it has been possible to observe it, is as follows :—

The contents of a hypha, usually one of small diameter, on approaching or on contact with a tracheid wall, become concentrated at the tip, as is shown by the deeper staining of this part of the hypha with the picro-anilin blue. (Plate III, figs. 1 and 3.) At this stage a change of shape takes place, a fine projection being visible, which may arise symmetrically as a fine prolongation of the tip, or may arise eccentrically, in which case the appearance resembles somewhat that of a pen nib in its holder. (Plate III, fig. 1.) The contents concentrate in the apex of this prolongation, which now grows out into a fine thread having dense contents. This prolongation at times is formed before the hypha is in actual contact with the tracheid. More commonly, only a very short point is formed before contact with a tracheid wall, and in some cases there is scarcely any constriction. The first visible sign of penetration is a V-shaped cut in the wall at the point of contact of the thread-like hypha. From this stage the action must be rapid, as, in spite of an examination of numerous sections, the intermediate stage between this and the emergence of the hypha on the other side was seldom seen. On emergence of the hyphal tip it resumes its original diameter in those cases where there has originally been some constriction. The bore-hole, at this stage, is approximately identical in diameter with the thread-like hypha. Often the same hypha can be seen to continue through many tracheids in succession in a direct line ; sometimes branching also takes place, and these branches in their turn penetrate the tracheids and bordered pits in all directions. (Plate III, figs. 7 and 8.) On emergence, the hypha may turn at right angles and run parallel and closely adpressed to the wall, sending off branches at right angles and penetrating the same tracheid at many points along its length. (Plate III, fig. 4.)

The bore-hole later becomes enlarged to many times the diameter of the hypha which formed it. Occasionally this is accompanied by a proportionate enlargement of the hypha. The portion of the hypha in the bore-hole, however, swells so that the hypha no longer shows any constriction. (See Plates III and IV.) The hour-glass shape of the bore-hole is not at all well marked on the first formation of the hole ; in the first instance being of even diameter ; it is during its enlargement that this shape is assumed. (Plate III, fig. 10.)

It was noticed in examination of sections stained by the picro-anilin blue method that around the bore-holes there was a well-marked halo of safranin, the wall of the tracheid adjacent to the hole being stained a much deeper red than the rest of the wall. This deeper staining of the wall was in evidence from the very first stage of penetration. (Plate III, figs. 3, 8 and 9 ; Plate IV, fig. 2.) Now, as there is little or no general delignification, it is suggested that, when the hyphal tip has made the bore-hole by solution of the cellulose of the wall, the lignin which was in that portion diffuses into the neighbouring portions of wall, increasing the lignin content in the immediate vicinity of the bore-hole.

This appears to be borne out further in that this halo is not even, but gradually suffuses outwards into the wall.

N.B.—It is possible that the substances allied to pectin are first attacked, seeing that from recent research there appears to be a gradual building-up from pectin to hemi-cellulose.

The smoothness of the sides of the bore-hole is difficult to account for, except on the supposition that the cellulose of the wall is altered or taken up by the fungus in advance, and that the actual penetration by the fine hyphal tip is thus made easy; this latter

process being, at any rate, partially mechanical. The secondary enlargement of the bore-hole, after penetration is complete, proves that enzyme action at this stage is not confined to the hyphal tip.

The most active hyphæ in penetration were found to be those of fine diameter which showed the medallion type of clamp. As has been mentioned previously, very few of the larger hyphæ appear to be associated with bore-holes (Plate IV, fig. 4) ; when this is the case they send off fine branches which bear medallions. (Plate III, figs. 4 and 10 ; Plate IV, figs. 1, 2 and 3.) This connexion between the presence of the medallion type of hypha and boring seemed so marked that it pointed to a tendency of hypha specialization for different functions. In agar cultures no medallion hyphæ have been seen, and this may account for the fact that little enzyme activity could be shown, using powdered mycelium from such cultures. Though too few experiments have been carried out as yet, further work now in progress may give more definite results.

THE FUNGUS GROWN ON ARTIFICIAL MEDIA

The following media were tried in order to discover the most suitable one on which to study the general growth and physiological relations of the fungus :—

1. *Czapek's Synthetic Medium*

$MgSO_4$	0·5 gm.
KH_2PO_4	1·0 gm.
KCl	0·5 gm.
$FeSO_4$	0·01 gm.
$NaNO_3$	2·0 gm.
Dextrose	30·0 gm.
Agar-agar	25·0 gm.
Water	1·0 litre.

2. *Peptone Glucose*

Glucose	40·0 gm.
K_2HPO_4	4·0 gm.
Peptone	4·0 gm.
$MgSO_4$	2·0 gm.
$CaCO_4$	0·25 gm.
CaCl	0·1 gm.
Agar-agar	15·0 gm.
Water	1·0 litre.

3. *Richards*

Cane sugar	50·0 gm.
KNO_3	10·0 gm.
KH_2PO_4	5·0 gm.
$MgSO_4$ $(7H_2O)$	2·5 gm.
$FeCl_3$	Trace.
Water, distilled	1·0 litre.

4. *Anti-Mould*

	Per cent.
Ammonium phosphate	0·2
Potassium phosphate	0·1
Zinc sulphate	0·4
Magnesium sulphate	0·05
Cane sugar	1·0
Agar-agar	1·0

5. *Glucose Potato Hard Agar-agar* (Thaxter)

Potatoes ..	200 gm., boiled for 1 hour in 1 litre of water and then made up to 1 litre.
Glucose	20 gm.
Agar-agar ..	30 gm.

6. *Malt Agar-agar*

Kepler's Malt Extract	20 gm.
Agar-agar	20 gm.
Water	1 litre.

7. *Prune Agar-agar*

Prune agar-agar	2 gm. of Prunes, simmer in water for 1 hour, and strain through cotton wool. Made up to 100 c.c., to which was added 2 gm. of agar-agar. Dissolved in autoclave and tubed.

8. *Xylose Medium* (6)

Xylose	2·5 gm.
Maltose	2·5 gm.
Ammonium phosphate..	0·5 gm.
Ammonium nitrate	0·5 gm.
$MgSO_4$	0·05 gm.
$FeSO_4$	0·025 gm.
$MnSO_4$	0·025 gm.
Agar-agar	10·0 gm.
Water	500·0 c.c.

For the trial a number of fungi were grown on these media at a constant temperature, and the growth of the colony on a Petri dish was measured each day to ascertain the medium most suitable for general use.

Results.—It was found that, for those wood fungi tested, growth was feeble on (2) Peptone Glucose, (1) Czapek's and (3) Richards, and scarcely any growth took place on (4), the anti-mould medium, which appeared to be toxic not only to moulds but to other fungi.

No. (8), the Xylose medium, proved to be the most satisfactory of the synthetic media. This was tried, as it had been stated that it encouraged fruiting, which incidentally was not proved by the writer to be the case with *M. lacrymans, P. vaporaria, Coniophora cerebella, Trametes serialis* and others which were grown on it.

The mycelial growth was fair, but it appeared in most instances to inhibit the formation of colour and other characteristic features useful in diagnosis, and therefore was not employed.

Very vigorous growth occurred on (5) Thaxter's Glucose Potato Hard Agar-agar, but this medium, being somewhat opaque, was found unsuitable for accurate measurement of the growth or for close observation ; further, it was inclined to encourage a too luxuriant vegetative development.

Good growth was obtained on (7) Prune Agar, but this varied too much in its *p*H in different samples, and so was employed only in those cases where a relatively high acidity was required either to keep bacteria in check (i.e., when isolating a fungus) or for certain fungi which require a high acidity for satisfactory growth.

It was found, on the other hand, that by using (6) Kepler's Malt Extract Agar a medium of standard *p*H could be obtained without correction, and the growth on this medium proved satisfactory.

It will be seen from the above remarks, that the use of synthetic media was found to be unsatisfactory for those lignicolous fungi which were tested, and this has been subsequently found to hold good for the majority of the Hymenomycetes.

The use of 2 per cent. Malt agar was on this account adopted throughout the present study where agar cultures were required.

Trametes Serialis Grown on 2 per cent. Kepler's Malt Agar-agar

The following observations were recorded :—

Growth.—Growth was moderately rapid at 20° C. up to 30° C. on Malt agar, a test tube slant (6 in. × ¾ in.) or a Petri dish (10 cm. diameter) being covered in about 11 days (see Table I and the chart on p. 14 for the temperature and rate of growth). The aerial and submerged mycelium grew at an approximately uniform rate of growth, but the aerial mycelium was not as a rule very pronounced. In slower-grown cultures there was a distinct halo effect due to the submerged mycelium being slightly in advance of the aerial. In this period the whole surface of the medium became covered with a somewhat sparse growth, which appeared patchy because of slight variations in the development of the aerial mycelium. The colour at this period was white, and in some cultures the appearance was somewhat sodden. Later, a greater development of aerial mycelium took place, though this was not the invariable rule, and in some cultures, especially Petri dish cultures in which the amount of nutrient agar was limited, this stage was followed by the formation of foliose fruit bodies and in the submerged mycelium by chlamydospore formation. In cultures where aerial mycelium development took place, this was usually more pronounced at the top end of the slant. The colour of this cotton wool-like aerial mycelium, on the ageing of the culture, often went through various tints of salmon to cinnamon, eventually becoming bistre brown, though in some instances no colour change took place.

Clamp connexions.—In the primary stage no clamps were observed, but in cultures a few days old these were found to be present, sparingly on the aerial mycelium, but more frequent on that of the submerged. Later, they were plentiful on both types. The clamp connexions were all of the simple type and no branching from them was observed.

Medallion hyphæ.—These have never been observed in cultures on artificial media.

Chlamydospore.—Formation commenced in a few instances in about three weeks after inoculation, but they were not found to be numerous until after six to seven weeks. No apparent connexion between their formation and the presence of clamp connexions could be detected, as had been noticed in the wood. (Plate V, fig. 4.)

Fruit bodies.—Those formed were mostly of the foliose type (Plate II, fig. 5). Plate II, fig. 3, shows a foliose type of fruit body commonly found on malt agar, though this was actually taken from a sawdust culture. Other forms, such as an irpiciform fruit body, also occurred. They resembled closely in many cases those figured by Falck[3] for *Lenzites abietina*. Occasionally, forms more of a minute dædaleoid type arose, but these did not mature fully.

Mycelium.—The aerial mycelium consisted of hyphæ mostly of uniform size, with numerous clamp connexions occurring at the septa. Branching took place frequently at angles of 45° to 90° and clamp connexions were present at many of the points of branching. In some instances, two to three clamps might be present at the junction of a branch. Finer hyphæ also occurred more sparingly interspersed amongst the general mycelium, and clamps were also present on these.

In addition, hyphæ of much larger diameter were interspersed in the general mycelial weft. These also showed clamp connexions and were thick-walled. No strand formation took place, though in a few instances there was shown a slight tendency to their formation. Chlamydospores, both terminal and intercalary, occurred, but were not nearly so numerous as in the submerged mycelium. The basidial fructifications arose either as scattered foliose plates, often somewhat gyrose or curved, on any part of the slant. In other cases they appeared in large confluent patches, resembling in every respect those which appear on the wood blocks.

Measurements in μ :—

Aerial Mycelium

Aerial hyphæ—1·5 to 3, average about 2 to 2·5.
Large hyphæ—up to 8.

Submerged Mycelium

1·5 to 8, average 3 to 4.

Chlamydospores

9 to 16 by 8 to 10.

Basidial Fructifications. (Plate V, fig. 5)

Basidia—

12 to 15 by 4 to 6 (occasionally 18μ × 6μ).

Sterigmata—

2 to 5 in length.

Basidiospores—

5 to 6 by 2·5 to 3.

Thus it will be seen that the measurements for the basidial fructifications are identical with those borne on the wood blocks. For photograph of basidial fructifications on agar see Plate II, fig. 5. For drawing see Plate V, fig. 5.

PHYSIOLOGY

Some of the matter under this heading was undertaken with the aid of Mr. W. P. K. Findlay, B.Sc., D.I.C., to whom the writer wishes to acknowledge his indebtedness.

Temperature and Rate of Growth.—A series of 10 Petri dishes, into which had been poured equal quantities of the 2 per cent. Kepler's Malt Agar-agar medium, were inoculated with small portions of inoculum containing actively-growing mycelium, all being taken from the same culture. Two dishes were placed in each of the temperatures tried in order to check the results, and the following temperatures were used, having a range thought likely to cover the optimum and minimum temperature favourable to growth, viz., 4°, 14°, 25°, 30° and 35° C. It was found that accurate measurements of the diameter of the colonies could be obtained by measurement from below, thus avoiding the risk of contamination through the raising of the covers. The results are set out in Table I and below.

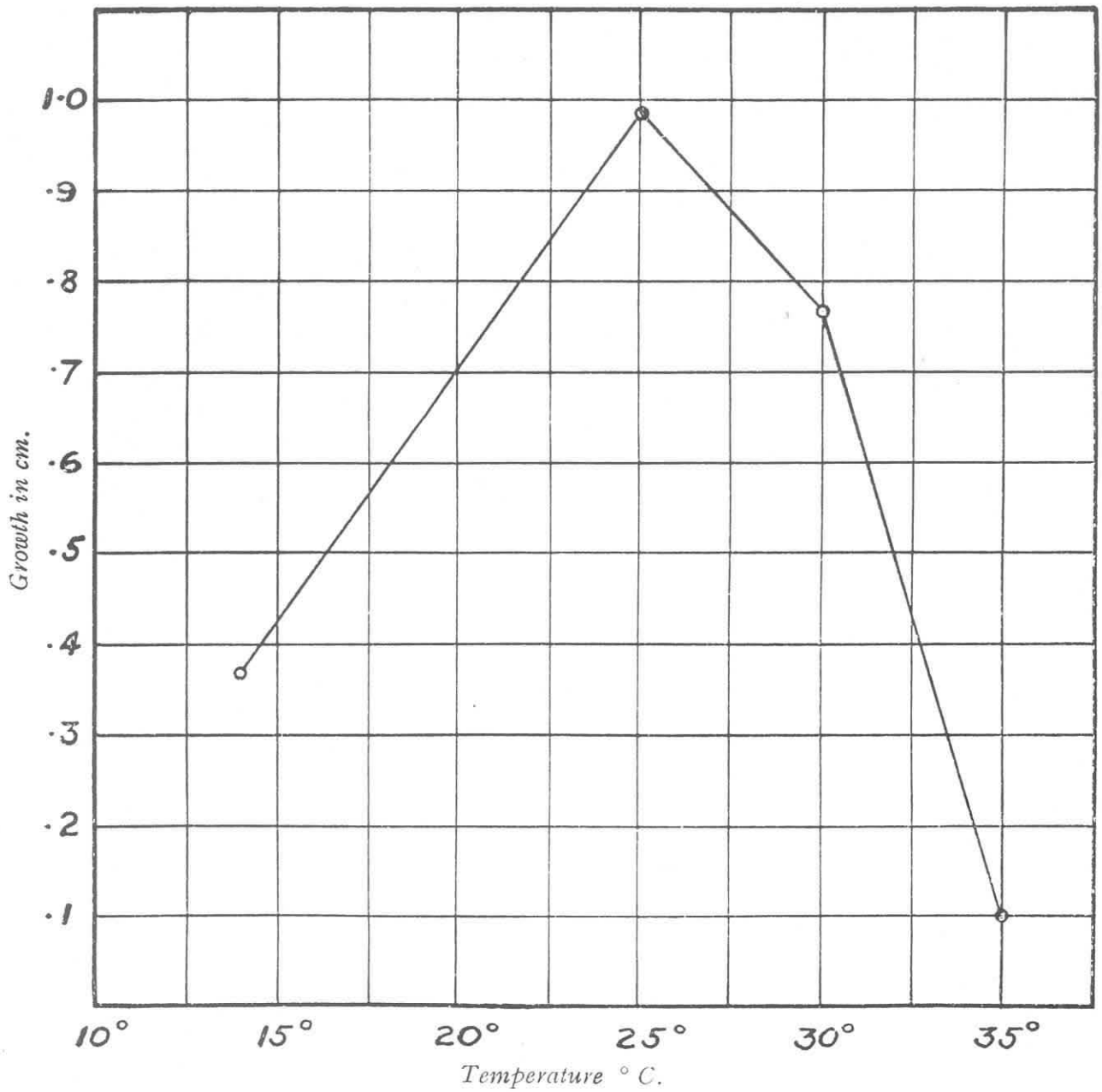

Chart showing rate of diameter growth per day.

From these it will be seen that the optimum temperature for growth lies near 25° C., none occurring at 4° C., and that at the other end of the scale only a very slight growth was observed at 35° C.

Insufficient incubators were available at the time to carry out experiments at higher temperatures to ascertain the lethal point, neither was it possible to experiment with temperatures between 4° and 14° C. It is hoped to deal further with the temperature relations in a paper to be devoted entirely to the physiology of wood-destroying fungi.

Growth on Acidified Medium.—Experiments were undertaken to obtain a rough idea as to the effect of acidity on growth. For this, concentrated HCl in one case and an N/10 solution in a second case were added to a malt agar, poured into Petri dishes and inoculated. It was found that acidity favoured growth, but not so markedly as in the case of many wood-destroying fungi such as *P. Schweinitzii*. It has been found, however, that with a few exceptions the *p*H values were not a matter of prime importance for these fungi, the range at which good growth is obtained being a wide one. They are, however, intolerant of an alkaline medium, and this has been made use of in experiments on preservatives. *Trametes serialis*, however, has been found to acidify its medium considerably, to the extent of bringing a medium which was neutral in reaction at the time of inoculation to a *p*H of approximately 3 in the course of one month's growth.

Enzyme Action.—A study of the enzymes present in the fungus mycelium is now being undertaken. So far, extracts of fungus mycelium grown on turnip juice have shown that the fungus possesses diastase and also has the power of breaking down cellulose. Sections of wood incubated at 35° C. in fungus powder extract, giving a less marked reaction to Schultze's solution than similar sections incubated in the autoclaved extract. No ligninase has as yet been demonstrated to be present.

Moisture in relation to Growth and Decay.—It has been noticed, when dealing with wood-destroying fungi, that both growth and rate of decay are, as a general rule, more affected by moisture than by temperature. Though resistance of these organisms to unfavourable conditions of humidity may be great and though they can exist, with an adequate aeration, under conditions far above the moisture content optimum, yet it has been usually found that they show an extreme sensitiveness to the degree of moisture, though this depends to some extent on the species.

It does not appear to be due so much to the actual quantity of water present, as to the correct balance between the amount of water and that of oxygen which enables or prevents the destruction of wood by the fungus.

Wood-destroying fungi can thus be very roughly divided into groups containing those which require very moist conditions and those which are tolerant of drier conditions. Thus, certain fungi are more or less restricted to the basements of houses and are rarely, if ever, found attacking roofs, e.g., *C. cerebella* (*puteana*), whilst others may be found in drier situations, but it cannot be said that there is any hard-and-fast line of demarcation between them, and it may be remarked that those fungi with a lower moisture requirement are usually able to thrive under moister conditions.

This question of the balance between the air and moisture content may be in part the deciding factor as to whether a facultative parasite attacks a tree or grows merely saprophytically in the surrounding humus or on dead wood. The presence of strains with varying degrees of virulence may also be partly dependent on such conditions, though it is not suggested that this is the ruling factor in the problem.

It would, however, be of great interest to have statistics showing the relations existing between soil moisture content and air humidity, and to compare these with those obtaining in the wood of the trees on the same site, both in cases where attack was prevalent and where the fungus, though present, appeared to be unable to establish itself as a parasite.

It is thought that, if such figures were available in the case of such prevalent fungi as *Armillaria mellea*, it might at least throw some light on the problem of its virulence.

EXPERIMENTS ON THE MOISTURE REQUIREMENTS OF *TRAMETES SERIALIS* ON SITKA SPRUCE BLOCKS

An experiment (No. 1) was started on March 21st, 1927, firstly, to ascertain the moisture conditions required for growth ; secondly, to find out how far figures for loss of weight after fungus activity could be utilized as a criterion of decay ; and, thirdly, how far these two could be correlated.

For experiment No. 1, 12 large test tubes, 12 in. × 3 in., were used, numbered respectively 1 to 12. Blocks of Sitka spruce, 15·7 × 3·5 × 3·5 cm., whose air-dry weight had been ascertained, were placed one in each tube, and varying amounts of water added to each (vide Table 2). These were then plugged with non-absorbent cotton wool and sterilized in an autoclave at 15 lb. pressure for 20 minutes. After cooling, they were inoculated from an actively-growing culture of *T. serialis* grown on malt agar. The inocula were of approximately equal size and taken from the same culture plate. The plugs were capped with cellophane and fastened by a rubber band to prevent the moisture drying out.

An external examination of this series was made on October 21st, 1927, the results of which are shown in Table 2, and in the same Table are given the remarks on the amount of decay which had taken place after seven months.

One of the difficulties encountered was the regulation of the moisture content, and it was apparent that, to secure this, and at the same time to avoid any possible poisoning effect on the fungus due to the accumulation of CO_2 formed in respiration, the experiments should be carried out in a self-regulating humidor ; but at the time this was not found to be possible. Experience has shown, however, that in the method employed aeration was not inhibited to a serious extent, and good growth, with no appreciable check during the course of the experiment, occurred. At any rate, the results have given a sufficiently accurate indication between certain ranges, which will afford data on which to base more accurately controlled experiments.

Apart from the numerous factors which are involved, the practical impossibility of obtaining samples of wood which will, under the same conditions, take up equal amounts of water, makes it doubtful whether extreme accuracy in regard to controlling these other factors would not be wasted.

A second experiment, No. 2, was started on August 8th, 1927, a rough idea of the moisture range required by the fungus having been gained by the first experiment. This No. 2 experiment was set up in a similar manner to that of No. 1, with the exception that, in place of a single undivided block being used, each block was divided into four equal-sized cubical portions, superimposed on each other, and the air-dry weight determined for each cube. Inoculations were made in the same manner as in the first experiment, except that the inoculum was placed at the junction of blocks two and three. The weights and amounts of water added are given in Tables 3A, 3B and 3C. The

experiment consisted of three series lettered respectively A, B, C. Each series consisted of five similar tubes, lettered W, X, Y, Z and CZ, of which the fifth was not inoculated, to act as a control.

The superimposed blocks in their turn were numbered from the bottom upwards 1, 2, 3, 4.

Series A was examined after four months, Series B after eight months, and Series C after twelve months. The moisture content of each block was then ascertained, and also the percentage loss of weight. A microscopical examination was made in the case of Series A to ascertain how far visual estimation of the extent of fungus activity might tally with the loss of weight estimation. Only one control was kept, as any alteration due to moisture alone would, it was thought, be most significant in that member of the series containing the maximum initial amount of water and space would have made it impracticable to furnish a control for each member of the series. (See Tables 3A, 3B, 3C.)

It is hoped later to make tests of mechanical strength after varying periods. Trials have already been carried out on small dimension samples by the Timber Mechanics Section of the Princes Risborough Research Laboratory, which show that this is quite feasible, provided a sufficient number of specimens are taken in order to arrive at a fair average.

Examination of Series A, B and C.—Each block of Series A was weighed after four months, allowed to dry, and re-weighed to obtain the air-dry weight. Oven-dry weights were taken in a few instances, but as it was desired to examine the entire block for fungus and the difference appeared to be a fairly consistent one, it was thought best in this instance to omit this procedure. Samples were taken at the commencement and the oven-dry weight for these samples ascertained before the wood was inoculated. A similar procedure was adopted in Series B and C.

The samples, on receipt, had an average moisture content of 10 to 12 per cent. From the figures obtained the percentage moisture content on the air-dry weight was calculated and the percentage loss of weight at the end of the experiment determined. (See Table 3.)

This loss of weight included loss due to autoclaving and other causes, but as the loss of weight due to autoclaving appeared to be a fairly constant factor, as is shown in the figures for the control, the significance of the figures for relative losses in weight due to decay is not affected. Each block, after obtaining the air-dry weight, was split longitudinally down the centre, and radial and tangential microtome sections prepared and stained. These sections were examined under the microscope for extent of fungus activity as judged by the presence of mycelium, bore-holes, etc., vide Table 4. For this, certain signs have been used in this Table to denote the extent of fungus activity, which, taken in conjunction with the remarks and loss of weight figures, give some idea of the extent of decay.

The figures giving percentage loss of weight agree closely with these somewhat indefinite estimates, and may therefore be taken to give an accurate measure of the extent of fungus activity. It is thought that if such figures were obtained for a wider range of moisture contents and for a larger number of samples, interesting comparative data would be obtained on which graphs representing fungus activity under different conditions, and also under similar conditions on different species of timber, could be constructed.

An interesting point emerges when the figures for the moisture content in tubes of series Z and CZ are compared, to which the same initial amounts of water had been

added. The one inoculated shows a considerably higher moisture content, this being due presumably to the action of the fungus in actually breaking up the wood and producing water, as well as by increasing the imbibing power of the wood. The samples were taken from the same block, yet certain differences might occur even in portions of samples so near together. The actual range of water contents is not sufficiently large for them to assume great significance, though the greatest loss of weight in this case occurred at a content of 43·7 per cent. of the total weight. It appears that a fair latitude is shown, though the optimum lies between 40 and 70 per cent. The difficulty of securing a sufficient range of water contents is seen by the small range of contents in spite of a large difference in the initial amount of water added, and it would appear that this could be better achieved in future experiments by placing the blocks horizontally in specially-designed flasks with a water chamber in the neck. Such flasks have already been made and are under trial. The blocks would be brought to a definite moisture content in a small kiln before being put in the flasks. Thus capillarity would not play so large a part, and by multiplying the number of specimens a greater range of moisture contents would be obtained.

It will be noted that the greatest loss in Series A occurred in AX and AY. In these, the moisture content for AX4 was 43·7 per cent. and in AY4, 46·5 per cent. Taking individual blocks, AX4 showed by far the greatest percentage loss, and AZ4 second with a moisture content of 59 per cent., AY4 being third with 46·5 per cent. and AW3 next with 60·4 per cent. It will also be noted that, with the exception of AW, where the greatest loss occurred in the block next to the top (AW3), in all others the optimum conditions appeared to have been found in the uppermost block, No. 4. This may be due partially to its better aeration, though the fungus appears to be for the most part an internal feeder.

These figures for loss of weight appear significant when compared with the remarks in Table 4, but can at present only be considered as tentative in view of the small number of samples taken.

The loss in AW4 appears unaccountably high for such a low moisture content of 23·9 per cent., but this is probably the maximum for this block, decay being caused initially when the grade of moisture contents was more even between the blocks in one tube.

The main significance of the figures lies in the percentage loss of weight, which was found to coincide remarkably well with the amount of active mycelium present in the block. As it is impossible to estimate quantitatively the amount of rot either by bore-holes or quantity of mycelium, the percentage loss of weight gives a useful measure of the amount of decay that has taken place.

The figures for Series B are given in Table 3 (*b*). The greatest loss in weight in any individual block was in block BY4, with a loss of 17·4 per cent., having a moisture content at this time of 47·46 per cent., or 89·75 per cent. on the air-dry weight. This maximum figure compares with that of 12·1 per cent. in Series AX4 at a moisture content of 43·7 per cent. The next in order was BZ4, with a loss of 16·76 per cent. Here the moisture content was only 20·15 per cent., or 25·3 per cent. on the air-dry weight. This can only be accounted for by presuming that the loss of weight occurred earlier, and, owing to the deep fissures extending right through the block, that loss of water was more rapid, so that during the latter period of the experiment decay had stopped, active growth and attack having only taken place during the earlier part of the period. A high loss of weight is also shown in W3, namely, one of 16·5 per cent., at a moisture content of 44·5 per cent., or 79·9 per cent. of the air-dry weight. Owing to the unavoidable variations in samples even when taken from the same block, entirely

consistent results are not to be expected, and only by means of a large number of repetitions can a fair average be gained on which it would be safe to draw definite conclusions.

Taking each divided block as a whole, it will be noticed that the greatest loss of weight occurred in Series B at average moisture contents of 62·73 per cent. and 55·76 per cent.; these figures represent, in all probability, a near approximation to the optimum for growth, though possibly the true figure lies slightly below this.

The loss due to autoclaving in CZ4 is unaccountably low. The other figures agree fairly closely with those in Series A.

It will be noted that in both cases the moisture contents for Z are higher than those for CZ, this being due presumably to the action of the fungus.

Figures for Series C are given in Table 4. Unfortunately, the control in this series became contaminated. In this series the highest loss of weight in any individual block was one of 32·2 per cent. in CW3, with a moisture content of 64·48 per cent., and 30·8 per cent. in CX3 at moisture content of 55·1 per cent.

On comparing the three series it will be noticed that there is a progressive loss of weight, CW3 and CX3 being almost entirely pulverised, only portions of the summer wood being left. It is not proposed to analyse these figures further, it being felt that fuller experimental data are required before it would be safe to attempt to generalize. When more figures are obtained with shorter intervals of time elapsing between the weighings, it is expected that the results will show a higher degree of consistency. In the above experiment the percentage moisture contents given are only those attained in the blocks at the end of the experiment. The action of the fungus in drawing up water and probably also manufacturing it in the process of attack; capillarity, and change in the imbibition power owing to decay, are all varying factors which will have to be taken into account.

When such data are available, it will be possible to represent the figures in graphic form and to correlate the various factors involved in the process of decay.

Summarizing the above, there is seen on the whole to be a progressive loss in weight due to decay, and this occurs within a wide range of moisture content, though all are above that of fibre saturation point.

It can also be seen that there is a tendency of the fungus to even up the moisture gradient through its power of producing water in the process of attack.

Much larger series kept under carefully-controlled conditions of humidity are indicated, and it is intended to carry out such experiments, using the same fungus and also another showing an external type of rot as a comparison.

CONCLUSION

Trametes serialis is a prevalent cause of brown pocket rot in Sitka spruce, its action being almost entirely confined to cellulose destruction.

The decay appears to be a very subtle one, as no signs of discolouration can be observed in many cases until the attack is in an advanced stage. In addition, hyphæ may extend a long way beyond the discoloured areas. It is therefore impossible in all cases to tell on inspection whether the wood is or is not infected. It has been found that hyphæ may be present the full length of any blocks up to 6 in. long after a period of four months, and that many of these blocks showed no visible sign of decay. It is thought probable that if longer samples had been employed this distance from the visible sign of the rot would have been far exceeded.

The mode of entry in the case of the experimental blocks appears to be usually at the cut ends, though the fungus is capable of entering through the sides. The hyphæ grow in the first instance longitudinally through the tracheids. On reaching an area containing the optimum moisture requirements they start to penetrate, probably by means of enzymes, in the first instance secreted at the tips of the actively-growing hyphæ. Pits for the most part appear to be ignored, and the hyphæ may penetrate through many walls in a straight line at right angles to the length of the tracheids. They may send off branches at intervals, which in their turn may penetrate any of the walls both of the tracheids and of the pits. This activity appears principally confined to the medallion type of hyphæ.

Under optimum conditions, the hyphæ may be found in masses, but often a few fine isolated strands may run the length of a tracheid, and then, on finding the conditions more favourable, may form a second rot pocket isolated from the first by what is apparently sound wood.

Brittleness is thought to be caused at an early stage after infection, and if the wood, appearing quite normal when taken from a log, shows any signs of brittleness or discolouration, it is advisable to follow Air Ministry practice and regard it with suspicion. This precaution is all the more needful as the fungus is capable of retaining its vitality and remaining dormant for considerable periods under air-dry conditions, and resuming its activities when conditions again become favourable.

For purposes such as joinery and cabinet work, where strength is not important, no particular attention might be paid to brittlenes. But it should be realized that such timber may be in the first stage of decay and that it is often impossible to detect the extremely fine hyphæ of some of the wood-destroying fungi without the aid of critical stains. Under favourable conditions the rot would continue to develop.

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APPENDIX

NOTE ON METHOD OF STAINING WITH SAFRANIN AND PICRO-ANILIN BLUE

1. Stain sections from water in a 1 per cent. aqueous solution of Safranin for about 30 seconds or upwards.

2. Wash out excess of stain with water, leaving the section slightly overstained.

3. Stain in picro-anilin blue, heating gently over a flame until the stain just begins to simmer, as when using Cotton Blue.

4. Rinse off excess of stain with water and then, if blue appears to be coming out too fast, finish off by differentiating with absolute alcohol, or quickly pass through different concentrations up to absolute alcohol.

5. Clear in clove oil, wash in Xylol or cedar wood oil, and mount in Canada Balsam.

The Picro-anilin Blue is made up as follows :—

Add 100 c.c. of saturated aqueous Picric Acid to 25 c.c. of saturated aqueous Anilin Blue. The hyphæ should stain a clear blue and the lignified tissue red.

Where the tissue has been badly attacked, this will also take on a blue colour, though even in this case the hyphæ are usually quite well differentiated.

TABLES

TABLE 1.—*Temperature and Rate of Growth*

Temperatures.

Date.	4° C.	14° C.		25° C.		30° C.		35° C.	
				Growth in centimetres.					
18th June ..	—	Inoculated.							
19th ,, ...	—	Growth started.		—		—		—	
22nd ,, ...	—	0·45	0·95	3·45	3·4	2·4	2·45	0·45	0·4
23rd ,, ...	—	0·60	1·40	4·60	5·10	3·1	3·1	0·70	0·4
24th ,, ...	—	*1·50	1·80	5·50	6·0	3·65	3·5	0·75	0·4
25th ,, ...	—	*2·65	2·0	6·9	7·1	4·5	4·2	0·80	0·55
26th ,, ...	—	*3·60	2·4	7·6	7·7	5·5	4·8	Incubator overheated.	
		2·0							
29th ,, ...	—	*5·8	3·2	8·5	8·5	8·5	7·7		
		2·5							
30th ,, ...	—	*6·4	3·8	—	—	—	8·8		
		3·0							
1st July	—	7·5	3·9						
2nd ,, ...	—	—	4·4						

Average rate of growth in cm. per diam. :—

4° C.	14° C.		25° C.		30° C.		35° C.	
0	0·33	0·42	1·04	0·95	0·78	0·75	0·15	0·05

Figures underlined show that the mycelium has covered the dish *x* — diameter of faint halo of fine hyphæ in the medium, these being in advance of the aerial hyphæ.

TABLE 2.—*Experiment* 1. *Details and report of results*

A. Ref. No.	B. Original Air-dry. Weight, gm.	C. Water in c.c.	D. Total Weight in gm. after seven months.	E. Air-dry. Weight, gm.	F. Per cent. Moisture content on D.	G. Per cent. Moisture content on E.	H. Per cent. Loss of Weight on B.	GENERAL REMARKS.	Remarks on External Growth of fungus in Moisture Experiments examined on October 21st, 1927, after seven months.
1	80·5	50	80·1	77·2	3·6	3·7	4·1	No rot pocket. Wood discoloured pink at base.	No growth except a commencement at point of inoculation.
2	81·5	100	98·8	76·1	23·0	29·9	6·1	Rot pocket easily seen, 4·0 cm. × 2·5 cm., and 2·5 cm. from base.	Growth moderate, reaching to just above half way, but unhealthy type of growth.
3	76·6	100	78·35	72·3	7·8	8·4	5·6	Very little rot, 0·5 cm. from base, 1·0 cm. × 1·5 cm. extent (— —), brittle.	Growth very sparse and weak, extending to half way up the blocks.
4	83·0	150	143·95	77·8	46·0	85·0	7·0	Rot pocket with deep cracks, 5·0 cm. from top, 4·0 × 3·0 cm.	Very good growth, extending to top of block. Mycelium profuse and start of strand formation.
5	87·0	200	156·6	82·2	47·3	91·0	5·5	Badly-rotted area situated 6·8 cm. from top, 3·5 × 2 cm. (+).	Sparse growth, reaching up to half way. Cushions of white aerial hyphæ formed at the upward limit of external growth.
6	78·1	200	166·1	72·4	56·4	129·4	7·3	Rot 7·5 cm., extending to top. Mycelium present all through, but sparingly at base.	Very good growth, reaching to top of block. Strand formation well developed. No fruit bodies.
7	87·6	250	183·5	81·3	55·7	123·2	7·3	Rot situated 3·5 cm. from top. Area rotted, 6·5 × 3·4 cm.	Sparse growth up to ¾ in. from bottom and cushions at top limit of growth. Slight suggestion of strand formation at base.
8	83·0	300	202·55	77·8	61·6	160·0	6·3	3·8 cm. to 4 cm. from top, total length of rot 9 cm.	Growth extending to top, where cushions had been formed on the sides of the block. No signs of strands and external growth on lower portion sparse.
9	79·4	300	196·9	74·7	62·1	163·6	5·9	No marked pocket—scorched and rotted externally—no rot in centre—rot extending 0·5 cm. from outside.	Identical with No. 8.
10	89·3	350	—	—	—	—	—	Wood for sampling—very badly rotted. Moisture content about 60 per cent.	Growth similar to No. 3, but more extensive, cushions covering top.
11	83·5	400	198·7	76·5	61·4	159·7	8·4	Scorched outside—no marked rot pocket. Very brittle and discoloured.	Very good mycelium growth and many plate-like fructifications formed on the sides of the block. (See Plate I, Figs. 3 and 4.)
12	77·2	400	171·45	75·4	56·0	127·4	2·5	Control—sound.	Control. No contamination.

TABLE 3 (*a*).—*Experiment* 2. *Loss of weight*

A. Ref. No. Series A.	B. Original Air-dry. Weight, gm.	C. Water added in c.c.	D. Total Weight in gm. after four months.	E. Air-dry. Weight, gm.	F. Per cent. Moisture content on D.		G. Per cent. Moisture content on E.		H. Per cent. Loss of Weight on B.	
W 1 ..	18·81	250	57·65	17·76	69·0	55·3	224·0	153·4	5·6	6·1
2 ..	18·74		54·41	17·75	67·7		207·0		5·3	
3 ..	22·98		53·50	21·25	60·4		151·5		7·5	
4 ..	20·39		25·21	19·18	23·9		31·28		5·9	
X 1 ..	23·37	300	62·15	22·28	64·5	60·2	178·8	162·6	4·7	7·0
2 ..	20·32		56·26	19·30	65·7		191·3		5·0	
3 ..	18·54		52·70	17·39	67·0		203·0		6·2	
4 ..	18·535		28·89	16·29	43·7		77·25		12·1	
Y 1 ..	24·25	350	66·1	23·05	65·1	61·6	187·0	171·3	4·94	6·4
2 ..	21·16		58·85	20·01	66·2		194·0		5·45	
3 ..	19·69		58·47	18·46	68·75		217·0		6·26	
4 ..	18·56		31·64	16·91	46·5		87·2		8·78	
Z 1 ..	21·82	400	59·37	20·87	69·25	66·3	224·0	200·8	4·33	6·1
2 ..	22·18		61·40	20·89	70·70		241·0		5·84	
3 ..	19·16		57·37	18·22	66·25		195·0		4·90	
4 ..	20·2		45·38	18·28	59·0		143·1		9·50	
CZ 1 ..	19·01	400	57·24	18·37	68·0	57·2	211·0	152·0	3·36	3·0
2 ..	18·54		54·11	17·93	66·75		201·8		3·26	
3 ..	20·065		47·08	19·46	58·75		142·1		3·25	
4 ..	19·045		28·45	18·63	35·45		52·75		2·18	

TABLE 3 (*b*).—*Experiment* 2. *Loss of Weight*

A. Ref. No. Series B.	B. Original Air-dry. Weight, gm.	C. Water added in c.c.	D. Total Weight in gm. after eight months.	E. Air-dry. Weight, gm.	F. Per cent. Moisture content on D.		G. Per cent. Moisture content on E.		H. Per cent. Loss of Weight.	
W 1 ..	21·21	250	59·52	19·70	66·75	48·04	202·0	124·63	7·09	8·6
2 ..	21·18		59·85	19·20	68·0		211·5		9·35	
3 ..	21·385		32·14	17·865	44·5		79·9		16·5	
4 ..	20·51		23·2	20·205	12·90		5·1		1·49	
X 1 ..	20·68	300	59·03	19·07	67·5	54·51	209·0	156·84	7·78	8·8
2 ..	20·16		58·24	18·52	68·2		214·0		8·17	
3 ..	21·335		55·12	19·49	64·7		183·0		8·68	
4 ..	19·4		21·30	17·545	17·62		21·35		9·75	
Y 1 ..	21·84	350	63·25	19·945	68·50	62·73	210·7	178·61	9·68	11·3
2 ..	20·25		59·22	18·550	68·75		219·0		8·40	
3 ..	20·525		54·76	18·555	66·20		195·0		9·60	
4 ..	19·63		30·73	16·21	47·46		89·75		17·40	
Z 1 ..	19·945	400	57·52	18·155	68·3	55·76	217·0	164·7	8·98	10·2
2 ..	18·945		56·52	17·30	69·25		227·0		8·675	
3 ..	20·345		55·23	19·06	65·35		189·5		6·325	
4 ..	18·375		19·15	15·295	20·15		25·3		16·76	
CZ 1 ..	22·555	400	62·6	21·84	65·1	57·98	186·0	149·73	3·18	2·3
2 ..	21·50		60·12	20·88	65·25		188·0		2·88	
3 ..	19·905		49·57	19·36	60·80		156·1		2·74	
4 ..	20·122		33·9	20·08	40·75		68·8		0·29	

TABLE 3 (*c*).—*Experiment* 2. *Loss of Weight*

A. Ref. No. Series C.	B. Original Air-dry. Weight, gm.	C. Water added in c.c.	D. Total Weight in gm. after twelve months.	E. Air-dry. Weight, gm.	F. Per cent. Moisture content on D.		G. Per cent. Moisture content on E.		H. Per cent. Loss of weight.	
W 1 ..	22·725	250	61·89	20·18	67·5	56·2	207·0	160·17	11·2	15·54
2 ..	21·06		61·56	18·95	69·25		225·0		10·0	
3 ..	20·13		38·255	13·64	64·48		180·5		32·2	
4 ..	22·485		26·86	20·52	23·55		28·16		8·75	
X 1 ..	21·395	300	58·88	19·54	66·8	51·49	201·3	51·49	8·68	12·81
2 ..	20·58		58·925	18·52	68·5		218·0		10·0	
3 ..	20·335		31·335	14·075	55·1		122·5		30·8	
4 ..	21·635		25·175	21·255	15·55		18·4		1·755	
Y 1 ..	21·47	350	57·83	19·115	67·0	57·95	202·0	169·7	10·9	12·14
2 ..	20·18		59·01	18·355	68·8		221·0		9·04	
3 ..	19·448		55·91	17·54	68·7		218·2		9·85	
4 ..	20·62		23·05	16·755	27·3		37·6		18·78	
Z 1 ..	21·025	400	59·16	19·68	67·0	55·54	200·5	166·64	6·4	7·64
2 ..	20·56		59·8	18·355	69·25		224·7		10·7	
3 ..	20·525		58·775	18·30	69·0		221·0		10·9	
4 ..	21·55		21·0	17·44	16·9		20·35		2·56	
CZ ..	—	—	Contaminated.							

TABLE 4.—*Symbols for fungoid activity*

eries A. Ref. No.	Extent of rot.	Remarks.
W 1	+−−	—
2	+−	—
3	++	—
4	+	—
X 1	+−	Mycelium sparse at top end and mostly near surface, which showed "scorching." Extent about 6 tracheids in—odd hyphæ beyond this.
2	+−	Mycelium confined to a few tracheids. Attack most at top.
3	++	Mycelium present throughout. Top end-badly rotted with many bore-holes. No visible sign of rot at lower end; but mycelium and bore-holes present sparingly.
4	+++	Rot all through.
Y 1	−−	Mycelium sparse and only at top. No bore-holes.
2	−−	Mycelium active at top end, not extending deeply. Only a few hyphæ at lower end near surface.
3	++	Mycelium abundant in top half and bore-holes numerous. Sparse at base, but showing some bore-holes.
4	+++	Mycelium present throughout. Less plentiful at top. Rot pocket at base partially formed and bore-holes numerous.
Z 1	−−	—
2	−−	—
3	−−	—
4	+++	—
CZ 1		—
2		—
3		No decay. Control.
4		—

No hyphæ .. .. −−−
Very few hyphæ and no bore-holes .. −−
Hyphæ not numerous, but a few bore-holes .. .. −
Moderate amount of hyphæ, bore-holes numerous. Distinct brittleness of wood .. .. .. +−
Active hyphæ plentiful, signs of decay visible without microscope +
Active hyphæ plentiful, decay pockets marked .. .. .. ++
Rotted badly, hyphæ very numerous and active .. .. +++

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PLATE I.

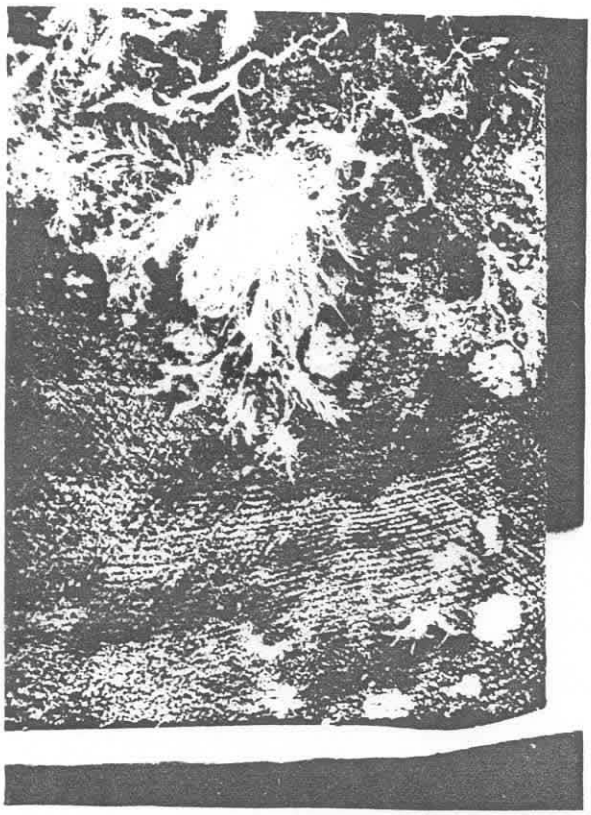


Fig. 1. Strands

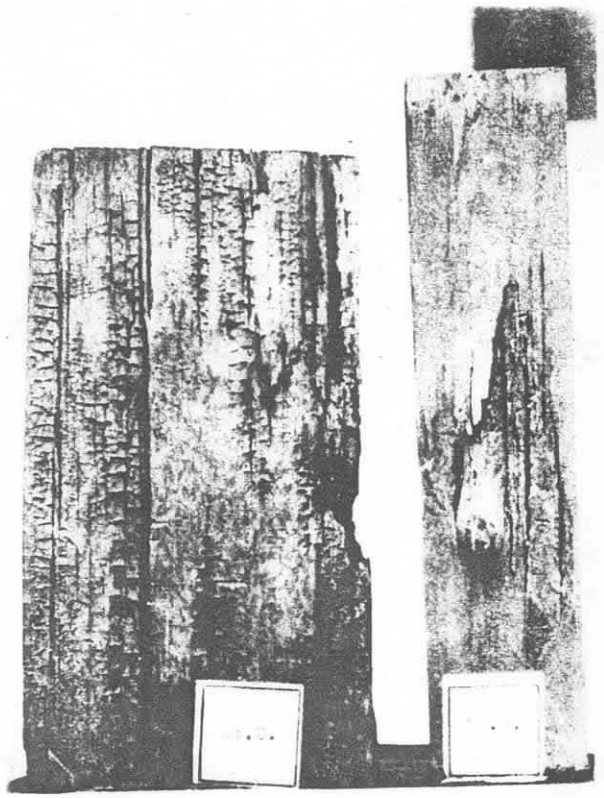
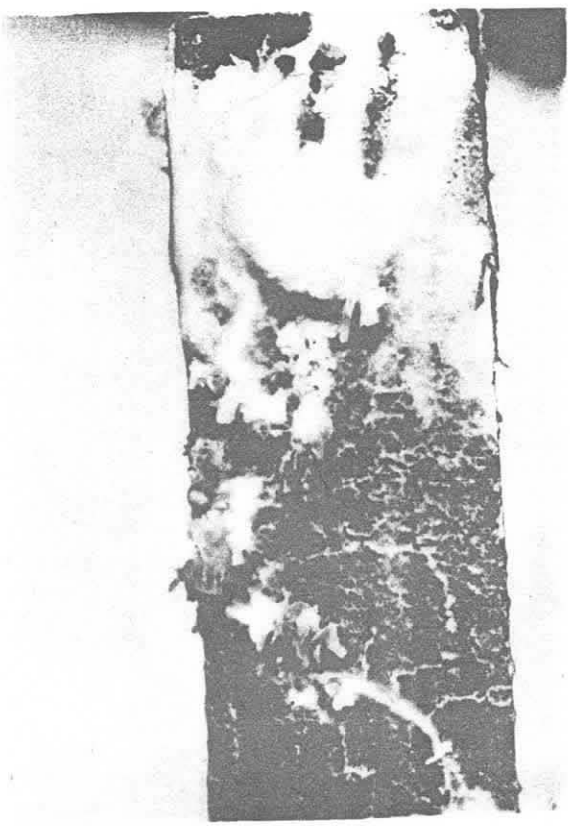
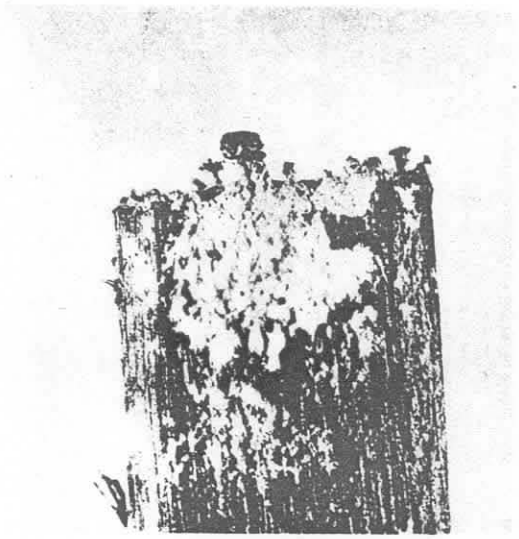


Fig. 2. Typical Decay
No 2. Natural *No 4 Artificially indu*

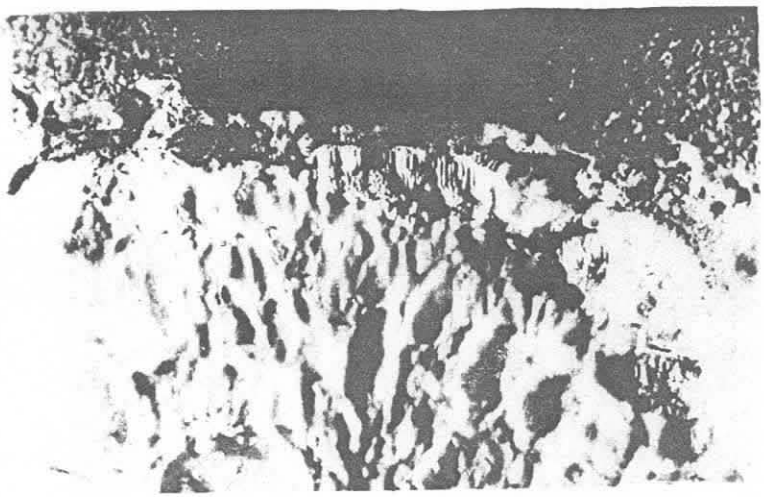


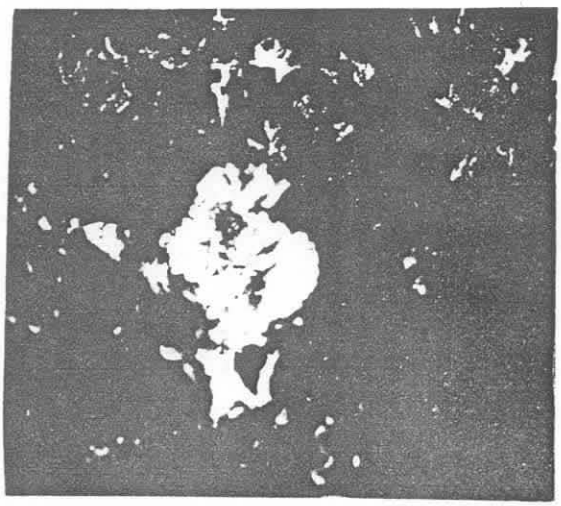
Figs. 3 and 4 Types of Sporophore.

PLATE. 2.



Figs. 1 and 2 Types of Sporophore on Wood





Figs. 3 and 4 Types of Sporophore on Sawdust

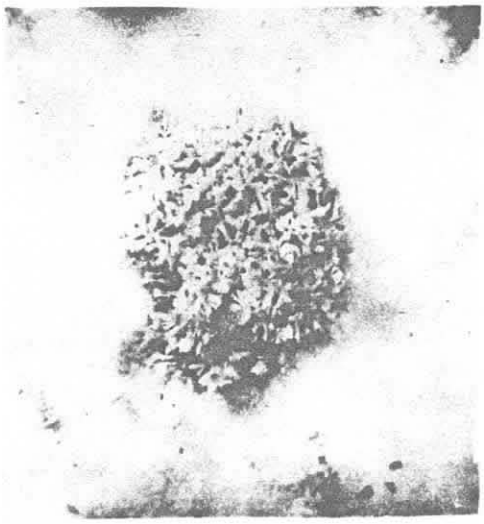


Fig. 5. Sporophore on Malt Agar.

PLATE 3.

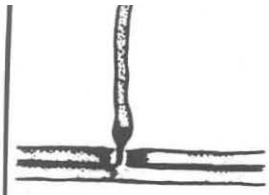


Fig. 1.

Fig. 2.

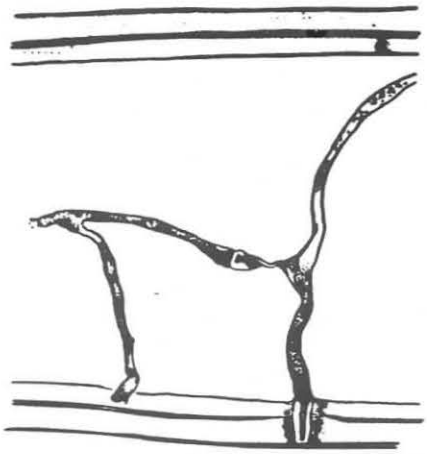


Fig. 3.

Fig. 4.

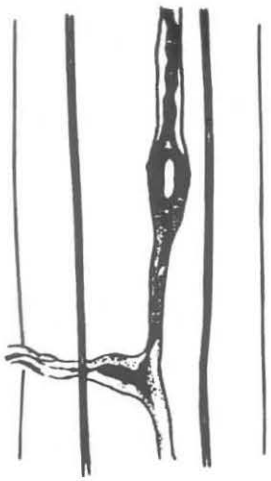


Fig. 5.

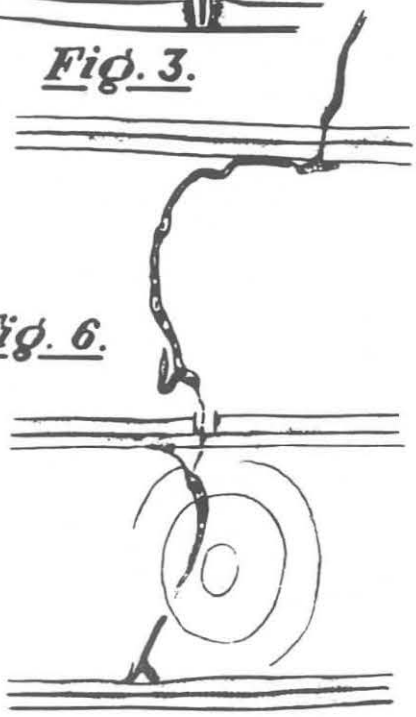


Fig. 6.

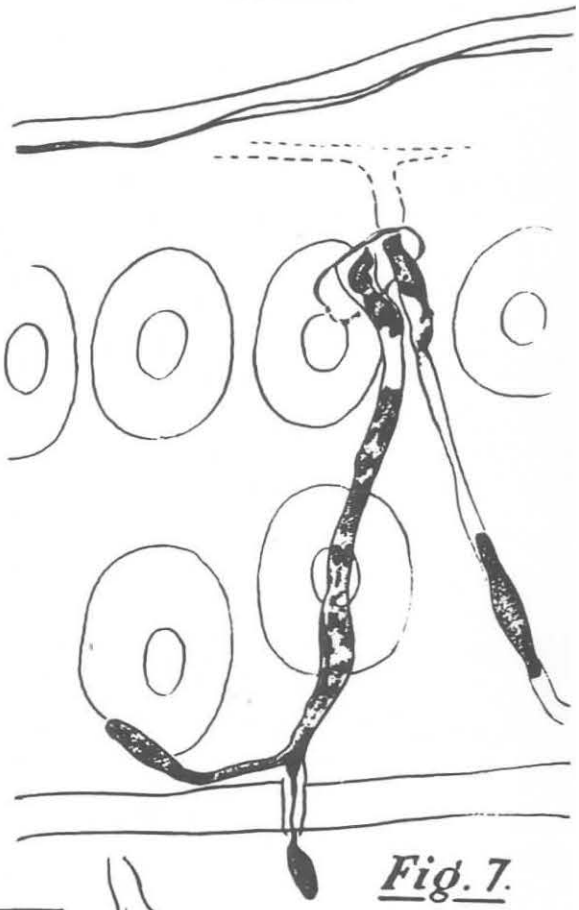


Fig. 7.

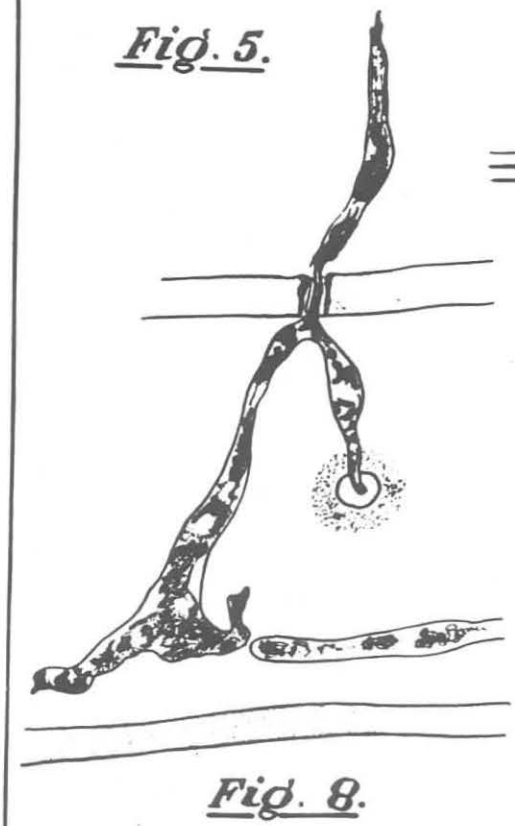


Fig. 8.

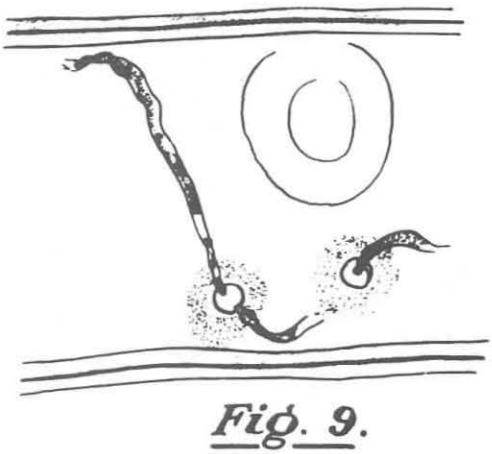


Fig. 9.

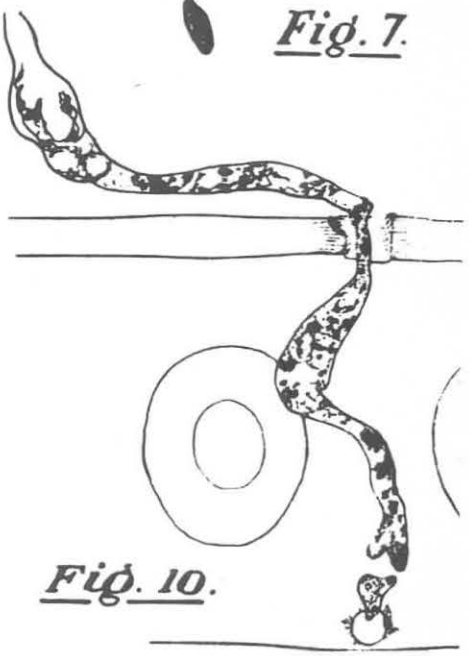


Fig. 10.

FIG. 1. EARLY STAGE OF PENETRATION OR SECTION NOT QUITE THROUGH MEDIAN PLANE. NOTE DENSE CONTENTS AT TIP x 900

FIGS. 2, 3, 4, 6, 7, 8, 9, 10 PENETRATIONS. NOTE FINE THREAD IN FIG. 6. AND LARGE BORE HOLE IN FIGS. 8, 9, 10. SLIGHT CONSTRICTION OF HYPHA WHICH USUALLY DISAPPEARS. WELL MARKED HALOS ARE SEEN IN FIGS. 3, 8, 9. FIG. 5 SHOWS A MEDALLION. FIG. 10. BORE HOLE SHOWING A TENDENCY TO HOUR GLASS SHAPE x 900 FIGS. 7 AND 10 ABOUT 1000

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PLATE. 4.

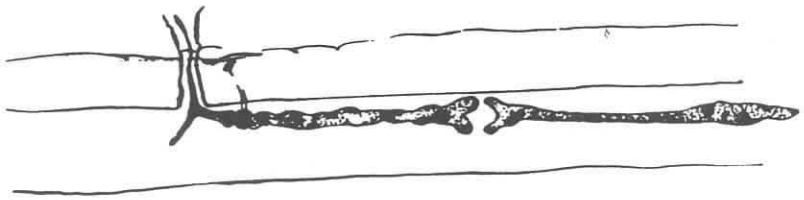

Fig. 1.

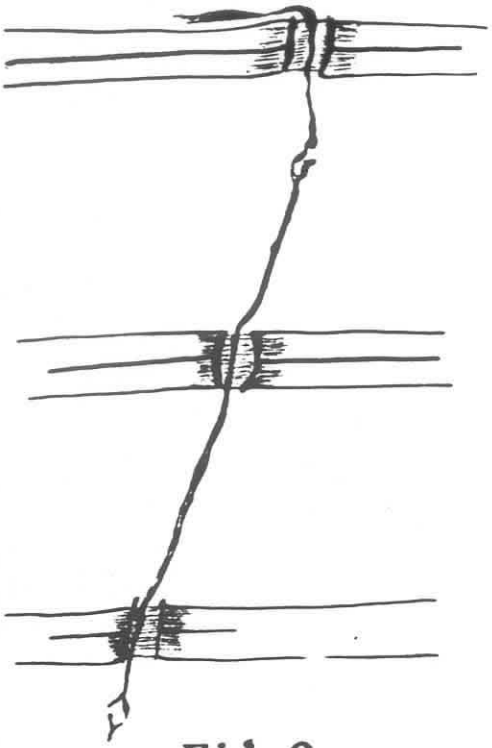

Fig. 2.

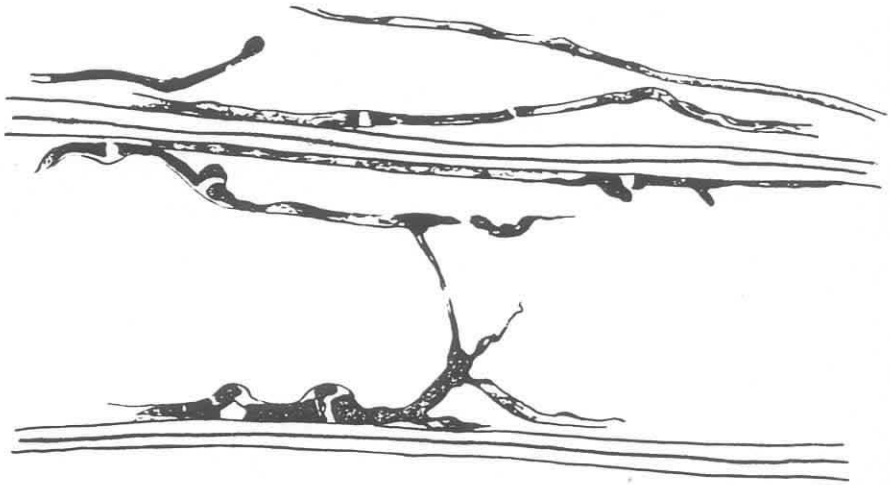

Fig. 3.

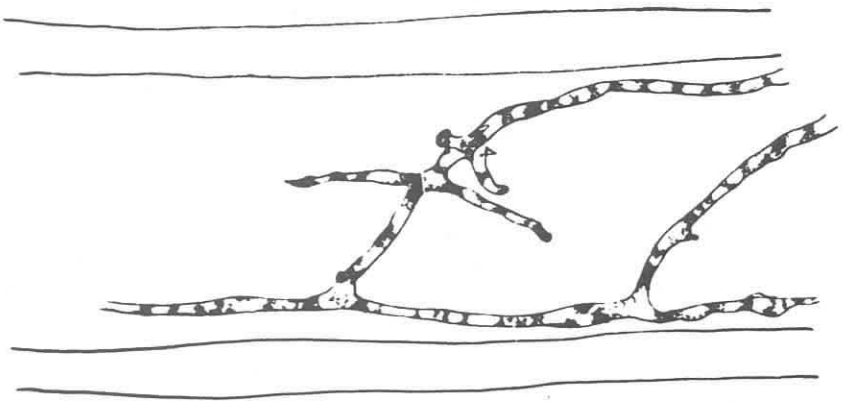

Fig. 4.

FIG. 1..... PENETRATION AND MEDALLION ABOUT 900 X

FIG. 2.... BORE HOLES IN CONSECUTIVE TRACHEIDS. NOTE HALOS AND MEDALLIONS. SLIGHTLY SHRUNK X 900

FIGS. 3 AND 4..... MYCELIUM IN TRACHEID. NOTE SIMPLE TYPE OF CLAMP AND NO BORE HOLES. X 900

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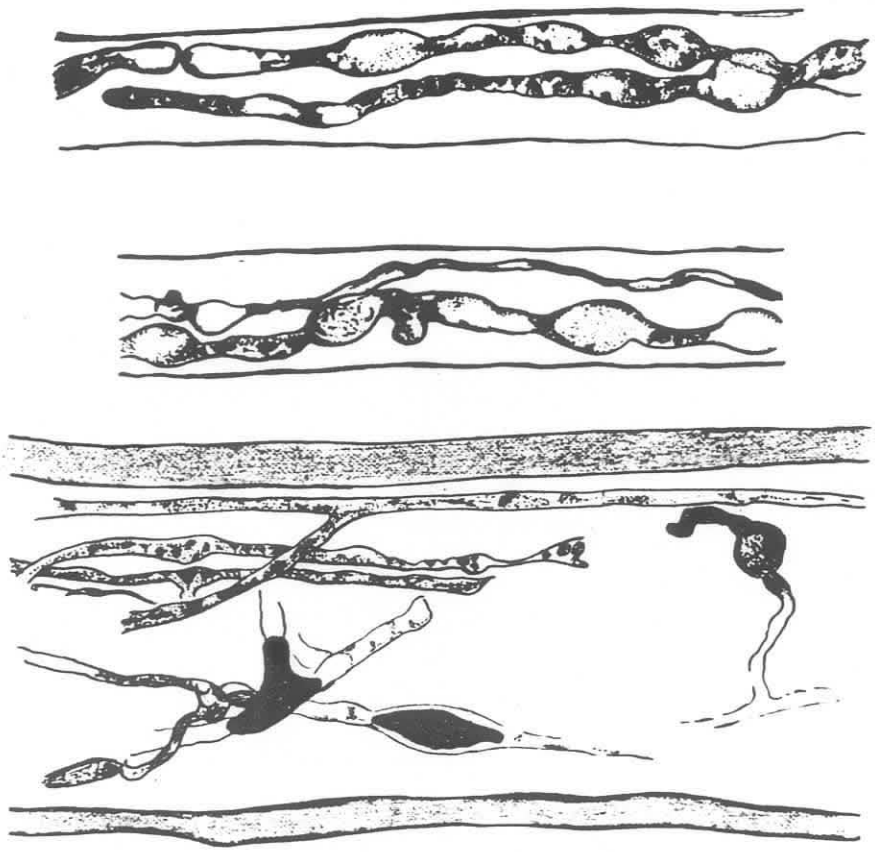
PLATE 5.



Figs. 1, 2 and 3.....Chlamydospores in Tracheids about 900 x

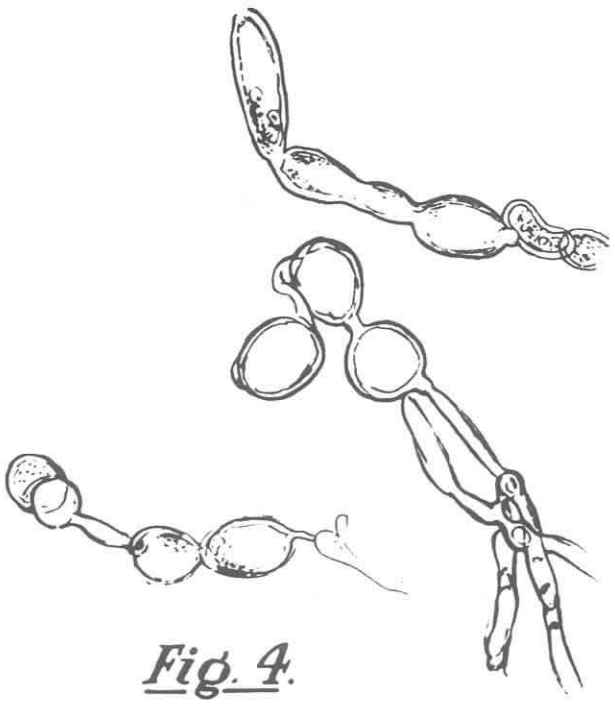


Fig. 4.
Chlamydospores in 2% Malt Agar Culture.
x 900

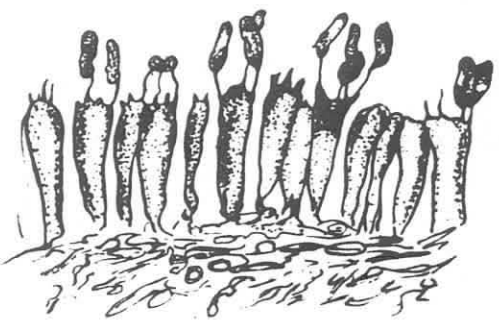


Fig. 5.
Portion of Hymenial Surface from a Foliose Fruit Body
x about 900

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PLATE 6

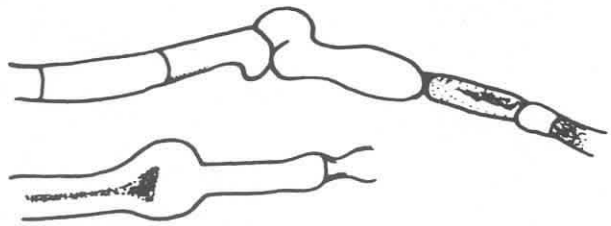

Fig. 1.

Fig. 2.

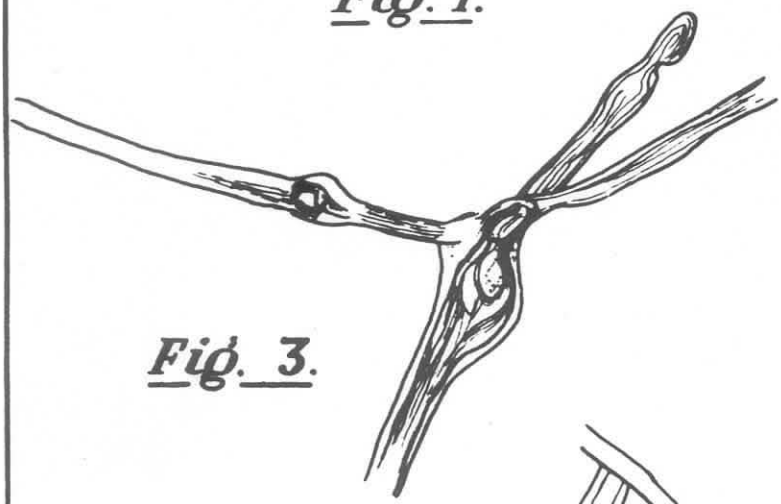

Fig. 3.

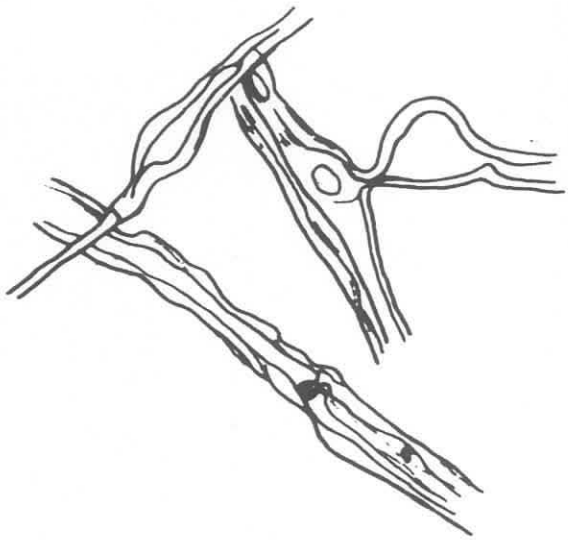

Fig. 4.

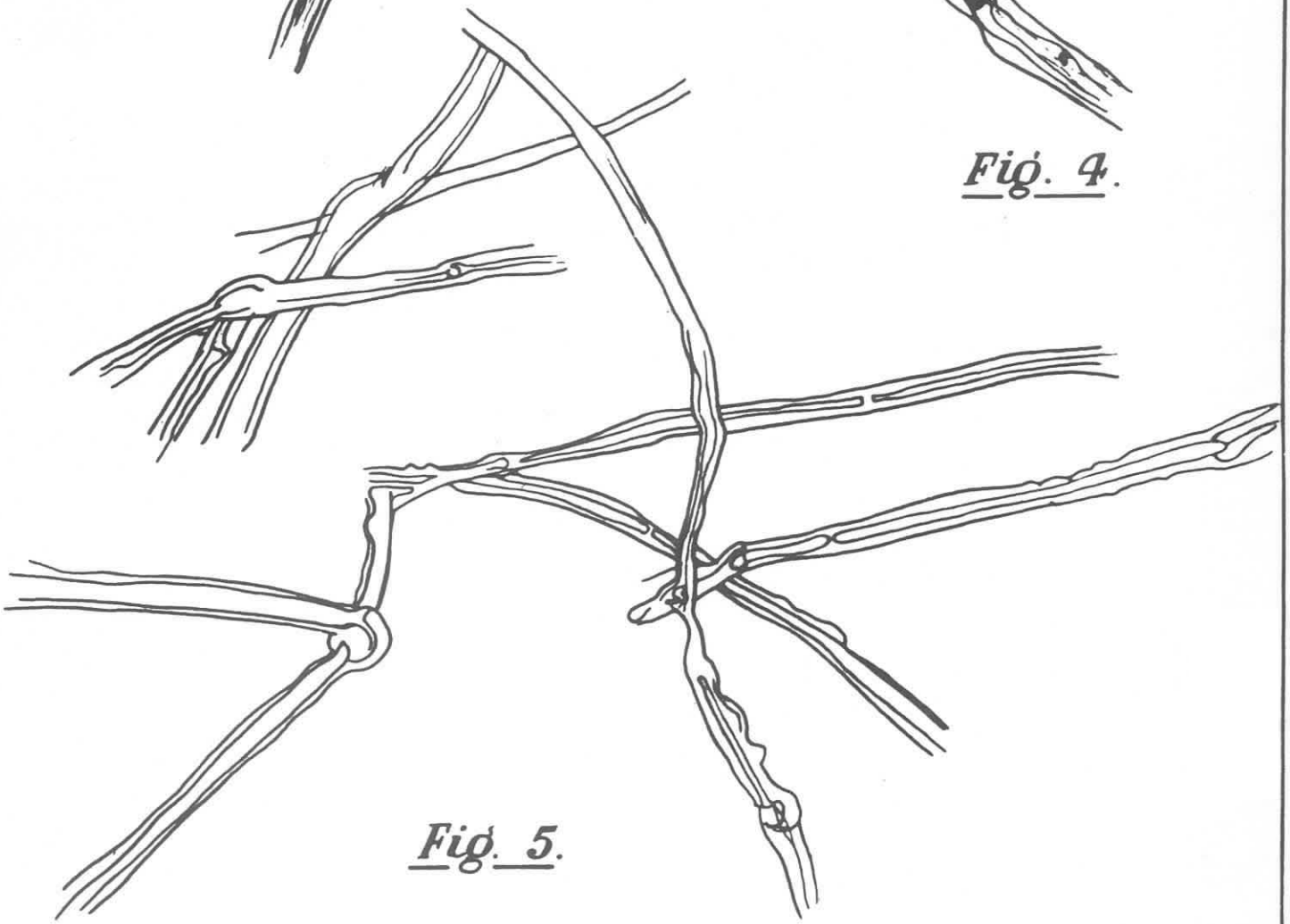

Fig. 5.

Strand Formation x about 1000

Note clamp in Fig 3 partially included in thickened wall.

Figs. 4 and 5 clamps totally included in wall.

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